

Supplementary Data

pH-Dependent random coil ^1H , ^{13}C , and ^{15}N chemical shifts of the ionizable amino acids: a guide for protein pK_a measurements

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Table S1 pH-dependent chemical shifts (ppm) of the ionizable amino acids in blocked Ac-Gly-X-Gly-NH₂ tripeptides^a

residue	nucleus type	nucleus	δ (HA)	δ (A)	$\Delta\delta$ (A - HA)	calc. δ (pH 7)
N-terminal amine: Alanine-amide (pK_a 8.23)						
Ala	¹ H	H (amine)	8.04			
		H α	4.10	3.51	- 0.59	4.07
		H β (methyl)	1.54	1.27	- 0.27	1.52
	¹³ C	C α	51.7	52.7	1.0	51.8
		C β (methyl)	19.3	22.9	3.6	19.5
		CO	176.0	184.6	8.5	176.5
¹⁵ N	N (amine)	40.4	33.8	- 6.6	40.0	
amide	¹ H	H ₂ N (Z)	7.24			
		H ₂ N (E)	7.82			
	¹⁵ N	N	106.1			
C-terminal carboxylic acid: N-acetyl alanine (pK_a 3.55)						
acetyl	¹ H	CH ₃ (methyl)	2.02	2.00	- 0.02	2.00
	¹³ C	CH ₃ (methyl)	24.3	24.7	0.4	24.7
		CO	176.8	176.1	- 0.7	176.1
Ala	¹ H	HN	8.35	7.94	- 0.41	7.94
		H α	4.33	4.12	- 0.21	4.12
		H β (methyl)	1.41	1.32	- 0.09	1.32
	¹³ C	C α	51.4	53.7	2.3	53.7
		C β (methyl)	18.8	20.1	1.3	20.1
		CO	179.6	183.0	3.4	183.0
¹⁵ N	N	110.5	115.7	5.2	115.7	

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	$\Delta\delta$ (A - HA)	calc. δ (pH 7)
Aspartic acid: Ac-Gly-Asp-Gly-NH₂ (pK_a 3.86)						
acetyl	¹ H	CH ₃ (methyl)	2.05	2.06	0.01	2.06
	¹³ C	CH ₃ (methyl)	24.4	24.5	0.1	24.5
		CO	177.8	177.8	0.0	177.8
Gly(- 1)	¹ H	HN	8.31	8.33	0.02	8.33
		H α (avg.)	3.94	3.95	0.01	3.95
	¹³ C	C α	45.4	45.4	0.0	45.4
		CO	174.8	174.6	- 0.2	174.6
	¹⁵ N	N	114.3	114.6	0.3	114.6
Asp	¹ H	HN	8.55	8.38	- 0.17	8.38
		H α	4.78	4.61	- 0.17	4.61
		H β (avg.)	2.93	2.70	- 0.23	2.70
		H δ 2 (carboxyl)	> 10			
	¹³ C	C α	52.9	54.3	1.4	54.3
		C β	38.0	41.1	3.0	41.1
		C γ (carboxyl)	177.1	180.3	3.2	180.3
		CO	175.8	176.9	1.1	176.9
¹⁵ N	N	118.7	120.2	1.5	120.2	
Gly(+1)	¹ H	HN	8.50	8.47	- 0.03	8.47
		H α (avg.)	3.91	3.90	- 0.01	3.90
	¹³ C	C α	45.1	45.2	0.1	45.2
		CO	176.9	177.3	0.4	177.3
	¹⁵ N	N	110.6	110.8	0.1	110.8
amide	¹ H	H ₂ N (Z)	7.10	7.10	0.00	7.10
		H ₂ N (E)	7.42	7.53	0.11	7.53
	¹⁵ N	N	107.2	107.6	0.4	107.6

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	$\Delta\delta$ (A - HA)	calc. δ (pH 7)
Glutamic acid: Ac-Gly-Glu-Gly-NH₂ (pK_a 4.34)						
acetyl	¹ H	CH ₃ (methyl)	2.05	2.06	0.01	2.06
	¹³ C	CH ₃ (methyl)	24.4	24.4	0.0	24.4
		CO	177.6	177.6	0.0	177.6
Gly(- 1)	¹ H	HN	8.31	8.31	0.00	8.31
		H α (avg.)	3.94	3.95	0.01	3.95
	¹³ C	C α	45.3	45.3	0.0	45.3
		CO	174.6	174.7	0.1	174.7
	¹⁵ N	N	114.3	114.4	0.2	114.4
Glu	¹ H	HN	8.45	8.57	0.12	8.57
		H α	4.39	4.29	- 0.10	4.29
		H β (avg.)	2.08	2.02	- 0.06	2.02
		H γ	2.49	2.27	- 0.22	2.27
		H ϵ 2 (carboxyl)	> 10			
	¹³ C	C α	56.0	56.9	1.0	56.9
		C β	28.5	30.0	1.5	30.0
		C γ	32.7	36.1	3.5	36.1
		C δ (carboxyl)	179.7	183.8	4.1	183.8
		CO	176.5	177.0	0.6	177.0
¹⁵ N	N	119.9	120.9	1.0	120.9	
Gly(+1)	¹ H	HN	8.53	8.55	0.02	8.55
		H α (avg.)	3.86	3.86	0.00	3.86
	¹³ C	C α	44.9			
		CO	176.7	176.8	0.1	176.8
	¹⁵ N	N	111.0	111.1	0.1	111.1
amide	¹ H	H ₂ N (Z)	7.08	7.09	0.01	7.09
		H ₂ N (E)	7.44	7.43	- 0.01	7.43
	¹⁵ N	N	107.2	107.3	0.0	107.3

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	$\Delta\delta$ (A - HA)	calc. δ (pH 7)
Histidine: Ac-Gly-His-Gly-NH₂ (pK_a 6.45)^c						
acetyl	¹ H	CH ₃ (methyl)	2.03	2.03	0.00	2.03
	¹³ C	CH ₃ (methyl)	24.5	24.5	0.0	24.5
		CO	177.4	177.4	0.0	177.4
Gly(- 1)	¹ H	HN	8.29	8.28	- 0.01	8.28
		H α (avg.)	3.88	3.87	- 0.01	3.87
	¹³ C	C α	45.4	45.3	- 0.1	45.3
		CO	174.3	174.3	0.0	174.3
	¹⁵ N	N	113.9	114.2 ^b	0.3	114.2
His ^c	¹ H	HN	8.55	~8.35 ^d	~ - 0.2	
		H α	~ 4.75 ^d	4.59	~ - 0.2	
		H β (avg.)	3.25	3.08	- 0.17	3.12
		H δ 2	7.30	6.97	- 0.33	7.04
		H ϵ 1	8.60	7.68	- 0.92	7.88
		H δ 1	>10			
		H ϵ 2	>10			
	¹³ C	C α	55.1	56.7	1.6	56.3
		C β	28.9	31.3	2.4	30.7
		C γ	131.0	135.3	4.2	134.3
		C δ 2	120.3	120.0	- 0.3	120.0
		C ϵ 1	136.6	139.2	2.6	138.6
		CO	174.8	176.2	1.5	175.9
		¹⁵ N	N	117.9	119.7 ^b	1.8
N δ 1	175.8		231.3 ^b	56	219.1	
N ϵ 2	173.1		181.1 ^b	8	179.3	
Gly(+1)	¹ H	HN	8.55			
		H α (avg.)	3.94	3.88	- 0.06	3.89
	¹³ C	C α	45.0	45.0	0.1	45.0
		CO	176.3	176.9	0.6	176.8
	¹⁵ N	N	111.0	111.5 ^b	0.5	111.4
amide	¹ H	H ₂ N (Z)	7.09	7.09	0.00	7.09
		H ₂ N (E)	7.53	7.42	- 0.11	7.44
	¹⁵ N	N	107.4			

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	$\Delta\delta$ (A - HA)	calc. δ (pH 7)
Cysteine: Ac-Gly-Cys-Gly-NH₂ (pK_a 8.49)						
acetyl	¹ H	CH ₃ (methyl)	2.06	2.07	0.01	2.06
	¹³ C	CH ₃ (methyl)	24.4	24.5	0.1	24.4
	¹³ C	CO	177.5	177.6	0.1	177.5
Gly(- 1)	¹ H	HN	8.32			
		H α (avg.)	3.98	3.97	- 0.01	3.98
	¹³ C	C α	45.4	45.4	0.0	45.4
		CO	174.6	174.2	- 0.4	174.6
	¹⁵ N	N	114.3	114.7 ^b	0.4	114.4
Cys	¹ H	HN	8.48			
		H α	4.56	4.28	- 0.28	4.55
		H β (avg.)	2.97	2.88	- 0.09	2.97
		H γ (thiol)	~ 2.0 ^e			
	¹³ C	C α	58.5	60.6	2.1	58.5
		C β	28.0	29.7	1.7	28.0
		CO	175.0	176.9	1.9	175.1
¹⁵ N	N	118.7	122.2 ^b	3.6	118.8	
Gly(+1)	¹ H	HN	8.58			
		H α (avg.)	3.93	3.92	- 0.01	3.93
	¹³ C	C α	45.0	45.1	0.1	45.0
		CO	176.6	177.1	0.5	176.7
	¹⁵ N	N	112.4	113.0 ^b	0.6	112.4
amide	¹ H	H ₂ N (Z)	7.09	7.09	0.00	7.09
		H ₂ N (E)	7.46	7.5	0.04	7.46
	¹⁵ N	N	107.3			

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	$\Delta\delta$ (A - HA)	calc. δ (pH 7)
Tyrosine: Ac-Gly-Tyr-Gly-NH₂ (pK _a 9.76)						
acetyl	¹ H	CH ₃ (methyl)	2.01	2.03	0.02	2.01
	¹³ C	CH ₃ (methyl)	24.4	24.5	0.1	24.4
		CO	177.3	177.4	0.1	177.3
Gly(- 1)	¹ H	HN	8.21			
		H α (avg.)	3.85	3.87	0.02	3.85
	¹³ C	C α	45.2	45.3	0.0	45.2
		CO	174.2	174.2	0.0	174.2
	¹⁵ N	N	114.1	114.3 ^b	0.2	114.1
Tyr	¹ H	HN	8.16			
		H α	4.55	4.49	- 0.06	4.55
		H β (avg.)	3.02	2.94	- 0.08	3.02
		H δ	7.14	6.97	- 0.17	7.14
		H ϵ	6.85	6.57	- 0.28	6.85
	¹³ C	H η (phenol)	~ 9.3 ^e			
		C α	58.0	58.2	0.3	58.0
		C β	38.6	38.7	0.1	38.6
		C γ	130.5	123.8	- 6.7	130.5
		C δ	133.3	133.2	- 0.1	133.3
		C ϵ	118.4	121.7	3.3	118.4
		C ζ	157.0	167.4	10.4	157.0
		CO	176.3	176.7	0.4	176.3
¹⁵ N	N	120.1	120.7 ^b	0.6	120.1	
Gly(+1)	¹ H	HN	8.44			
		H α (avg.)	3.82	3.83	0.01	3.82
	¹³ C	C α	45.0	45.0	0.1	45.0
		CO				
¹⁵ N	N	112.4				
amide	¹ H	H ₂ N (Z)	7.07			
		H ₂ N (E)	7.21			
	¹⁵ N	N	107.1			

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	$\Delta\delta$ (A - HA)	calc. δ (pH 7)
Lysine: Ac-Gly-Lys-Gly-NH₂ (pK _a 10.34)						
acetyl	¹ H	CH ₃ (methyl)	2.05	2.05	0.00	2.05
	¹³ C	CH ₃ (methyl)	24.4	24.4	0.0	24.4
		CO	177.4	177.4	0.0	177.4
Gly(- 1)	¹ H	HN	8.31			
		H α (avg.)	3.94	3.94	0.00	3.94
	¹³ C	C α	45.4	45.3	- 0.1	45.4
		CO	174.5	174.6	0.1	174.5
	¹⁵ N	N	114.4	114.5 ^b	0.1	114.4
Lys	¹ H	HN	8.40			
		H α	4.34	4.30	- 0.04	4.34
		H β (avg.)	1.82	1.78	- 0.04	1.82
		H γ	1.44	1.36	- 0.08	1.44
		H δ	1.68	1.44	- 0.24	1.68
		H ϵ	3.00	2.60	- 0.40	3.00
		H ζ (amine)	7.52	~ 1 - 2 ^f	~ - 6 ^f	7.52
	¹³ C	C α	56.4	56.9	0.4	56.5
		C β	32.8	33.2	0.3	32.9
		C γ	24.7	25.0	0.4	24.7
		C δ	28.9	33.9	5.0	28.9
		C ϵ	42.1	43.1	1.0	42.1
		CO	177.0	177.5	0.5	177.0
¹⁵ N	N	121.0	121.7 ^b	0.7	121.0	
	N ζ (amine)	32.7	~ 25.2 ^g	~ - 7.5 ^g	32.7	
Gly(- 1)	¹ H	HN	8.50			
		H α (avg.)	3.91	3.90	- 0.01	3.91
	¹³ C	C α	44.9	44.9	0.0	44.9
		CO	176.8	176.8	0.0	176.8
¹⁵ N	N	111.0	111.1 ^b	0.1	111.0	
amide	¹ H	H ₂ N (Z)	7.07			
		H ₂ N (E)	7.45			
	¹⁵ N	N	107.2			

Table S1 - con't

residue	nucleus type	nucleus	δ (HA)	δ (A)	$\Delta\delta$ (A - HA)	calc. δ (pH 7)
Arginine: Ac-Gly-Arg-Gly-NH₂ ($pK_a \sim 13.9$)						
acetyl	¹ H	CH ₃ (methyl)	2.05			2.05
	¹³ C	CH ₃ (methyl)	24.4			24.4
		CO	177.5			177.5
Gly(- 1)	¹ H	HN	8.31			8.31
		H α (avg.)	3.94			3.94
	¹³ C	C α	45.4			45.4
		CO	174.6			174.6
	¹⁵ N	N	114.4			114.4
Arg	¹ H	HN	8.43			8.43
		H α	4.36			4.36
		H β (avg.)	1.84			1.84
		H γ	1.65			1.65
		H δ	3.21	~ 3.04	$\sim - 0.17$	3.21
		H ϵ (guan.)	7.19			7.19
	¹³ C	H η (guan.)	6.64			6.64
		C α	56.3			56.3
		C β	30.6			30.6
		C γ	27.0			27.0
		C δ	43.3			43.3
		C ζ (guan.)	159.5	~ 163.0	~ 3.5	159.5
	¹⁵ N	CO	176.7			176.7
		N	120.7			120.7
		N ϵ (guan.)	84.8			84.8
	N η (guan.)	$\sim 71^h$			$\sim 71^h$	
Gly(- 1)	¹ H	HN	8.51			8.51
		H α (avg.)	3.91			3.91
	¹³ C	C α	44.9			44.9
		CO	176.5			176.5
¹⁵ N	N	111.0			111.0	
amide	¹ H	H ₂ N (Z)	7.07			7.07
		H ₂ N (E)	7.46			7.46
	¹⁵ N	N	107.3			107.3

Table S1 - cont

- ^a Recorded at 25 °C with 50 mM NaCl and 5% D₂O, unless indicated. Reported are the fit pK_a values and end point chemical shifts δ (ppm) of the of the acid (HA) and conjugate base (A) forms, along with the chemical shift change upon deprotonation ($\Delta\delta$; negative is upfield) and the predicted shift at pH 7. The estimated errors are ± 0.05 for pK_a values (± 0.1 for arginine), ± 0.02 ppm for ¹H nuclei, ± 0.08 ppm for ¹³C, and ± 0.06 ppm for ¹⁵N. Blank values indicate not determined. Prochiral proton shifts are averaged. The C-terminal amide protons are assigned assuming Z/E as upfield/downfield.
- ^b Recorded in 99% D₂O and corrected for the deuterium isotope shift.
- ^c Data for neutral histidine is an average of ~80% N^ε2H and ~20% N^δ1H tautomers.
- ^d Estimated from (Kjaergaard et al. 2011).
- ^e From the BioMagResBank (Ulrich et al. 2008).
- ^f From (Takayama et al. 2008).
- ^g From (Andre et al. 2007).
- ^h From ¹³C₆/¹⁵N₄-L-arginine (Table S2).

Table S2 pH-dependent chemical shifts (ppm) of $^{13}\text{C}_6/^{15}\text{N}_4\text{-L-arginine}$ ^{a,b}

nucleus type	nucleus	δ (HAH) pH ~ 7	δ (HA) pH ~ 11.5	δ (A) pH > 15	$\Delta\delta$ due to amine (HA - HAH)	$\Delta\delta$ due to guan. (A - HA)
		$\alpha\text{-COO-}$ $\alpha\text{-NH}_3^+$ guan+	$\alpha\text{-COO-}$ $\alpha\text{-NH}_2$ guan+	$\alpha\text{-COO-}$ $\alpha\text{-NH}_2$ guan		
^1H	HN (amine)	7.81				
	H α	3.77	3.26	3.19	- 0.51	- 0.07
	H β (avg.)	1.89	1.60		- 0.29	< - 0.1
	H γ	1.67	1.60		- 0.07	< - 0.1
	H δ	3.24	3.19	3.00	- 0.06	- 0.19
	H ϵ	7.22				
	H η	6.67				
^{13}C	C α	57.2	58.4	58.6	1.2	0.2
	C β	30.3	34.4	35.2	4.1	0.9
	C γ	26.6	27.2	28.1	0.6	1.0
	C δ	43.3	43.8	44.3	0.5	0.5
	C ζ	159.6	159.6	163.5	0.0	4.0
	CO	177.1	185.8	186.1	8.7	0.2
^{15}N	N (amine)	40.6	33.2	33.6	- 7.5	0.4
	N ϵ	84.4	85.6	91.5	1.2	5.9
	N η	71.5	71.2	93.2	- 0.3	22

^a Recorded at 25 °C for 100 mM $^{13}\text{C}_6/^{15}\text{N}_4\text{-L-arginine}$ with 5% D₂O, 1 mM DSS, and initially 50 mM NaCl (final KOH > 10 M). Blank values indicate not determined due to rapid HX or spectral overlap. The $^1\text{H}^\beta$ shifts are averaged. Due to bond rotations, the two $^{15}\text{N}^\eta$ and four $^1\text{H}^\eta$ yield broad signals. The data for neutral arginine are tautomer averaged.

^b Tabulated are the fit chemical shifts for the two-step sequential titration from pH 7 to 15.25 of the α -aminium (pK_a 9.15 \pm 0.05) and then the guanidinium (pK_a 13.9 \pm 0.1) moieties in the context of the α -carboxylate anion. The estimated fitting errors are \pm 0.05 ppm for ^1H nuclei, \pm 0.1 ppm for ^{13}C , \pm 0.15 ppm for ^{15}N amine and $^{15}\text{N}^\epsilon$, and \pm 0.3 ppm for $^{15}\text{N}^\eta$.

Supplemental References

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